Methanol Institute

July 3, 2002

Dr. Michael Shelby
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Dear Dr. Shelby:

The Methanol Institute appreciates this additional opportunity to comment on the draft final report of the Expert Panel on the Developmental and Reproductive Toxicity of Methanol. As you know, the Methanol Institute provided written comments on the initial draft report (Sections 1-4) on September 5, 2001, and Dr. John Clary of BioRisk provided expert oral testimony on behalf of the methanol industry at the October 15, 2001 public meeting.

Our attached comments provide a few additional remarks directed at Sections 1-4, while the majority of our comments have focused on the summary and conclusions contained in Section 5. We believe that Sections 1-4 of the Expert Panel report provide a useful summary and analysis of the available data on the reproductive and developmental effects of exposure to methanol. However, further clarification seems needed with several of the conclusions stated by the Expert Panel. We believe the Panel inappropriately employed rodent data in assuming a developmental effect to exposure of low levels of methanol to pregnant women. Further, the Panel's arbitrary setting of an unnecessarily low "safe level" for methanol is not justified by the available data.

We also remain concerned – and somewhat mystified – by the undue length of time it took the CERHR to release its final draft report. The report's Section 5 was written during an open public meeting in October, but it took until May to publish the Federal Register notice. This delay will add to the public's perception that the CERHR's consensus process appears to have been breached with this report.

While the comment period for this final draft report closes on July 8th, we would strongly urge the CERHR to include the presentations and discussions

that will take place on July 9th, during a half-day session titled "Methanol – Is it a Developmental Toxicant?" at the Tox Forum in Aspen, Colorado, as part of the public record for this report. We anticipate that this program will provide the National Toxicology Program with substantive guidance for preparing the final report on this topic.

The charter of the CERHR does not take into account the very real economic implications of its findings, but as the trade association for the global methanol industry, we are obligated to do so. In reviewing the current and future potential for consumer exposure to methanol, the report cites the use of a wide array of methanol-containing products (windshield washer fluid, paints, varnishes, and Sterno heaters), dietary exposure from fruits and diet soft drinks, and the potential for the broader use of methanol fuels in motor vehicles. Further, methanol is a leading candidate hydrogen carrier fuel for a range of fuel cell technology applications.

We were quite pleased to be involved in this process. We also have high hopes that the Expert Panel's conclusions ultimately will provide guidance in determining the potential for developmental or reproductive effects from exposure to methanol. Such guidance would be useful in helping the methanol industry to limit the potential for any harmful exposures. As the Panel's preliminary conclusions stand today, we find the ultimate utility of this report to be less than we had hoped. In choosing an arbitrary "safe level" for methanol and raising concerns about the potential for developmental effects in women exposed to "high levels" of methanol based on rodent data, the Panel's conclusions may serve as a detriment to a better understanding of this issue.

We would certainly appreciate the opportunity to keep this dialogue open as the NTP and NIEHS prepare their final report for publication. We would be happy to meet with representatives of NTP/NEIHS to discuss our concerns.

Sincerely,

John Lynn
President & CEO

Enclosure

COMMENTS ON

FINAL DRAFT

NTP-CERHR EXPERT PANEL REPORT ON

REPRODUCTIVE AND DEVELOPMENTAL

TOXICITY OF METHANOL

JULY 3, 2002

Methanol Institute

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CERHR EXPERT PANEL REPORT ON METHANOL

The final draft report incorporates most of the suggested corrections and answers to the comments submitted by the Methanol Institute in September 2001 on the first draft of Sections 1-4. Sections 1-4 were well done. Each study was reviewed in depth, giving all possible important experimental details and the results. In addition, the strengths and weakness of each study were discussed, as well as, the utility (adequacy) for the evaluation processes. A few comments on the final draft are addressed to Section 1-4, but the majority of the comments on this final draft are addressed to Section 5, the summary and conclusions.

SECTION 1-4

In Section 2 Page 46, additional comments should be made about Table 2-10. At the end of the first paragraph, Table 2-10 is discussed in comparing actual blood methanol levels at 1000 and 5000 ppm in different species. It would be useful also to discuss the predicted values from the Perkin's PBPK model (1995) (see Page 29 in the report) as a measure of how good the predictions in the different species were when compared to the actual data.

Table 2-10 also contains estimated dose in mg/kg. The estimated dose in mg/kg should be discussed in the text, and used to compare species response under different conditions. This estimate is very helpful in comparison between species that are exposed for different lengths of time to different airborne concentrations. The estimate dose in mg/kg correlates much better than airborne concentrations, with increasing blood methanol levels. In various areas of the text where difference in response in the same species are noted (at the same airborne concentration), a reference to the estimated dose may make these differences in response easier to understand. For example, both Nelson et al (1985) and NEDO (1987) exposed rats to 5000 ppm, but the NEDO exposure was three times longer (22.7 vs 7 hours per day) (see Page 66-67), and therefore the estimated dose was three times higher in the NEDO study. The estimated dose in mg/kg would be useful in

cases where NOAEL and LOAEL are noted in different studies and species.

It seems that an understanding of estimated dose in mg/kg in all circumstances (different species, concentrations, and length of exposure) would strengthen this document and its conclusions, and may also be very useful for risk assessment purposes. It is strongly recommended that estimated dose in mg/kg be included in all discussion of results.

Top of Page 65. The statement is made that the blood methanol levels did not appear to reach saturation at any dose in the Rogers et al study (1993). This statement needs some clarification since the blood methanol levels increased five fold for a two fold increase in concentration (1000-2000 ppm), supporting that the catalase enzyme was saturated.

Top of Page 68 under prenatal studies a NEDO study (1987) in monkeys is mentioned, but no results are given. A statement is given that ILSI reached some conclusions about this study, but no comments about the CERHR Expert Panel conclusions are mentioned. The Burbacher et al (1999) study, which also covers prenatal evaluations, is not mentioned here in the section devoted to prenatal effects.

On Page 71 reference numbers 97 and 142 are Stern et al, not Weiss et al.

SECTION 5 CONCLUSIONS

Page 108 : "the panel also concluded that there was sufficient evidence that methanol was a developmental neurotoxicant in rodents..." This statement found in the conclusion appears to be stronger than the data from Section 3.2.2 indicates.

The conclusions about developmental neurotoxicity are based on several studies (Pages 68-72). On Page 69, the study by Infurna and Weiss (1986) reports that the oral dose was high (above the lethal dose in humans) and only one dose was used. The study noted an increase in latency to effect nipple attachment, but this study was considered to be of limited utility for the CERHR process (Paragraph 4 Page 70). In the study by Stanton et

al (1995) (Page 70), the dose was over two times higher (15,000 ppm - 6100 mg/kg) than the dose (2500 mg/kg) in the Infurna and Weiss (1986) study, but no developmental neurotoxicant effects were noted. There was concern the group size may have been too small to pick up effects, but the much higher dose should also have produced a greater effect.

In the studies by Weiss et al (1996) the exposure was to 4500 ppm (1444 mg/kg) from day 7 of gestation to day 21 of age. The offspring had blood methanol levels more than twice the dams. However, in stark contrast to the Infurna and Weiss (1986) study, no increase in latency to effect nipple attachment was noted. The results do suggest some gender related difference in methanol pups in a test that assess cognitive and motor function. Since the animals were exposed for 21 days after birth it is not clear that the effects noted are developmental or just a neurotoxic response in very young animals. In addition, the last sentence Paragraph 6 Page 72 points out that "an experimental design that does not permit evaluation of dose response adds uncertainty to the utility of the finding." A review by an HEI committee (Paragraph 2 Page 72) states that care must be taken **not** to ascribe too much significance to these results.

The results of these postnatal rat studies are slight, variable, and are largely not reproducible. The lowest dose (mg/kg) had effects while the highest dose (four times higher) had no effects. Clearly the results fail to support the statement found in the conclusion. The conclusion about developmental neurotoxicity should be modified to state the finding of subtle or suggestive evidence, not sufficient evidence. The evidence of developmental neurotoxic effects in the primate is described as *subtle, but not definitive adverse effect.* The same wording should apply for the rodent data.

The statement in the middle of the first Paragraph on Page 108 that "2000 ppm or greater... can cause... cleft palate, exencephaly and skeletal malformation," is incorrect. Only an increase in cervical ribs was observed at 2000 ppm. The other effects noted were observed at higher doses.

The middle of the Paragraph on reproductive toxicity on Page 108 states "(that blood methanol level was not reported but speculated by the panel to be 700-1000 mg/l based on other studies)," is incorrect. The blood methanol level in rats exposed to 5000 ppm for seven hours is 1000-2170 mg/l (see Table 2-10). In the NEDO study, cited exposure was three times as long per day or roughly equivalent to 15,000 ppm for seven hours (blood methanol level 3169-3826 mg/l) (see Table 2-10).

The conclusions in this report raise several significant concerns. The CERHR Expert Panel concluded that methanol may be a developmental toxicant to pregnant women exposed to high levels of methanol. The conclusion was based primarily on data in rodents and several broad assumptions. It is important to assess the relevance of this conclusion by taking a closer look at the data and the assumptions that are raised. These assumptions include: (1) methanol is the proximately toxicant; (2) effects are associated with high blood methanol levels; (3) the metabolism is similar in rodents and humans; (4) rodents are good models for methanol in humans; and (5) blood methanol is a useful indicator of exposure. There is no data in humans to support any of these conclusions, but pregnant women are somehow considered as a susceptible subpopulation in this report.

A 'safe blood methanol level" (10 mg/l) was also established in the report's conclusion. The basis for arriving at this "safe level" is never clearly articulated.

RELEVANCE TO HUMANS

There appear to be three key studies addressing developmental toxicity, two of these studies are in rodents (Nelson et al 1985, Roger et al 1993) and the third is in primates (Burbacher et al 1999). The developmental conclusion is based on rodent data that is assumed to be relevant to humans.

In Section 5, the CERHR Expert Panel concluded that "The available rodent data are assumed to be relevant for humans because of the known similarity among species in

early embryonic development, and that the experimental models used to evaluate methanol teratogenesis (i.e., in vivo and in vitro studies with rodents) have been shown to be useful for known human teratogens."

However, the report states on Page 82 that: "Given what is known about the saturation of methanol metabolism under high exposure conditions the relevance of the high dose rodent developmental studies for human risk assessments is uncertain and needs careful consideration by the CERHR Expert Panel."

The CERHR Expert Panel appears to have adopted a default position in assuming relevance, rather than dealing with an understanding of metabolism and species difference as indicated on Page 82. Surprising, no other support is offered for the CERHR Expert Panel position in the conclusion.

This is a weak argument in the case of methanol, where the relevance of rodent data to humans is questionable at best. For example, NTP never conducted a bioassay on methanol in rodents because NTP concluded that differences in metabolism between rodent and humans (rats accumulate methanol in the blood at toxic doses, while humans accumulate formate) made rodents poor models for humans. Different enzymes (catalase in rodents and alcohol dehydrogenase in humans and primates) control the first step in the metabolism of methanol to formate. Pharmacokinetic models predict that differences in blood methanol concentrations are not large in rodents and humans exposed to methanol at low exposure concentrations (NEDO 1987, Ward et al. 1997, Horton et al. 1992).

Actual data where a comparison is made on a estimated dose in mg/kg supports this prediction. At higher concentrations, species blood methanol levels responses are vastly different. At 1,000 ppm, pharmacokinetic models predict the blood methanol concentration in mice is three to seven fold greater than that in humans, while exposures to 5,000 ppm will result in blood methanol levels fully 13-18 fold greater in mice than humans (Perkins et al. 1995). Saturation of catalase is demonstrated in rodents by an exponential increase in blood methanol, while there is no evidence of formate

accumulation in rodents.

Alcohol dehydrogenase is not the rate limiting steps in the metabolism of methanol in humans or primates. The rate-limiting step in humans is demonstrated by a significant increase in blood formate, that is the step that converts formate to carbon dioxide.

Methanol is considered by the report as the most likely proximate toxicant, but the mechanism of action of methanol in rodent developmental studies is unknown. If it is unknown, how can the rodent data possibly be relevant to humans? Developmental effects are associated with high blood methanol (>500 mg/l), and not formate levels. In humans, formate levels increase and cause serious toxicity (blindness, death), well before significant increases in blood methanol are seen.

Developmental effects are associated with the catalase, not alcohol dehydrogenase metabolism, and with the levels above saturation of catalase. The impact of saturation of the enzyme catalase on the developmental response is unknown. In rodents, the enzyme (catalase) is saturated at high doses resulting in high blood methanol level (all effect levels in the rodent studies are above a saturating dose). The normal role of catalase is to help protect against toxicity induced by oxygen radicals (reactive oxygen). The addition of catalase to cell cultures has been reported to inhibit teratogenicity caused by several teratogenic agents such as phenytoin (Winn and Wells 1999), benzo(a)pyrene (Laposa et al 2000), and arsenicals (Hunter et al 1999), suggesting a role for reactive oxygen in these specific teratogenic responses. Inhibition of catalase has been reported to produce a significant increase in malformation in cultured mouse embryos (Bauman et al 1996, Poon et al 1998)

To compare species response to methanol, a total daily delivered dose/estimated dose can be estimated in all species based on minute ventilation, length of daily exposure, and airborne concentrations used (see Table 2-10 and attached graph). The total daily delivered dose (estimated dose) is based on mg/kg bw per day. ***The developmental effects seen in rodents are only seen at doses (mg/kg) several fold above the lethal dose***

in humans (see attached graph).

A more informed conclusion about relevance could be reached if all the data were fully considered.

"SAFE LEVEL"

Another problem with the report is the ambiguity regarding how a "safe blood methanol level" (below 10mg/l) was derived. Does it assume that humans are more sensitive than the most sensitive rodent specie? Was the safe level selected simply because it is above the blood methanol level seen at normal dietary intake or inhalation exposure at the PEL? Under some circumstances normal occupational exposure could exceed the safe level if inhalation exposure was at or close to the PEL and some skin exposure occurred and or dietary intake of fruit was high. In a human chamber study exposure to 200 ppm for six hours resulted in a blood methanol level of 7-8 mg /l. Dietary intake of fruit was restricted and no skin exposure occurred. Immersion of a hand in methanol for 16 minutes has been shown in humans to result in blood methanol levels higher than the "safe level" (Franzblau et al 1995). If you take six hours inhalation exposure at 200 ppm, add 8 ounces of orange juice (600-ppm methanol) in the diet and some skin exposure the blood methanol levels could be higher than the "safe level."

The conclusions about reproduction are weak and unsubstantiated. They suggest that more data is needed before safety can be assured at specific blood methanol levels.

GENERAL COMMENTS

We believe that Sections 1-4 of the Expert Panel report provide a useful summary and analysis of the available data on the reproductive and developmental effects of exposure to methanol. Our concern is with many of the conclusions discussed in Section 5 of the report, particularly the four bullet points found on Page 111.

First, the Panel finds a "minimal" concern that low blood methanol concentrations associated with dietary and work exposure to methanol may result in developmental toxicity to humans. While we can take some comfort that this concern is just "minimal," not every reader of this report will note this semantic distinction. The line between the various definitions of "concern" established by the CERHR are so fine, that most readers (and potentially policy-makers) will simply take a broad-brush approach and reach a far more onerous conclusion. It may not be the purpose of the Expert Panel to conclude that drinking a can of diet soda or eating an orange may lead to a developmental effect – and the data in Sections 1-4 clearly does not provide any justification for setting such an arbitrary "safe level" – but that may be the unfortunate conclusion that some draw. The attempt to set a "concern" classification, and boil down a wealth of significant research into a bullet point does more harm than good. It would be much better and more accurate to state that dietary and worker exposure to methanol is not at all likely to lead to developmental effects.

The second bullet point contends that exposure to high levels of methanol may be a developmental toxicant to pregnant women simply is not supportable by the data. The relevance of rodent methanol data to humans is the key issue. The data on methanol supports the position that the rodent is not a good model for humans in this case. Even assuming that rodent data is relevant, when the biological basis for this determination "remains unknown" (Page 110), is too far a stretch. The equivalent level of exposure to humans at which rodents showed weak developmental effects would be fatal. The Panel appears to have ignored its own admonition to recognize species differences in methanol metabolism and toxicity.

With the third bullet point, the Panel appears to have found that low concentrations of methanol will not have any reproductive effect on males. Although, here again, the classification of "negligible concern" following the CERHR guidelines could remain open to subjective interpretation. The Panel also felt compelled to temper this statement by stating that "high, acutely toxic doses of methanol might affect male reproduction." Once again, the levels of methanol exposure consistent with the rodent data this statement

is attributed to would be fatal in humans, providing little concern for effects on offspring.

In the final bullet point, the Panel found insufficient data to assess whether methanol is a reproductive hazard in females. The data actually did not indicate any significant findings of reproductive hazards to females from methanol exposure. The issue is not so much a lack of data, but that the data failed to provide any reasonable level of concern.

The Panel also sought to identify several "Critical Data Needs" on Pages 111-112. We would agree that the CERHR/NTP should attempt to contact the Japanese NEDO to obtain a full and translated copy of their important work on methanol. We also concur that the Burbacher et al. study suffers greatly from a lack of valid statistical analysis. Without such a rigorous evaluation of this oft-quoted primate study, it is extremely difficult to reach any consensus on what this study proves or disproves.

The purpose of the CERHR Expert Panel was to gather the best available data on methanol effects, and produce a reasonable set of conclusions. In our previous written comments to the Panel and our oral testimony provided at the public forum, we have attempted to further strengthen the already solid analysis completed in Sections 1-4. This has been our first, and we hope not our last opportunity to comment on the Panel's conclusions articulated in Section 5. We were quite pleased to be involved in this process. We also have high hopes that the Expert Panel's conclusions ultimately will provide guidance in determining the potential for developmental or reproductive effects from exposure to methanol. Such guidance would be useful in helping the methanol industry to limit the potential for any harmful exposures. As the Panel's preliminary conclusions stand today, we find the ultimate utility of this report to be less than we had hoped. In choosing an arbitrary "safe level" for methanol and raising concerns about the potential for developmental effects in women exposed to "high levels" of methanol based on rodent data, the Panel's conclusions may serve as a detriment to a better understanding of this issue. As new markets for methanol develop in areas such as emerging fuel cell technologies that offer significant economic, energy security and environmental benefits, the goal of our industry in providing a safe product become even more important.

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RESPONSE IN RATS COMPARING TOTAL DAILY DELIVERED DOSE AND BLOOD METHANOL TO LETHAL DOSE IN HUMANS (NELSON ET AL.)

EXPOSURE CONDITIONS Ppm - 7 Hrs	BLOOD METHANOL (mg/l)	DAILY DOSE (mg/kg)	Ratio of total dose in mice to lethal dose humans (300-1000 mg/kg)
5000	1000-2170	1969	~2 - 6
10000	1840-2210	3738	~4- 12
20000	5250 -8650	7476	~7 - 25

RESPONSE IN MICE COMPARING TOTAL DAILY DELIVERED DOSE AND BLOOD METHANOL TO LETHAL DOSE IN HUMANS (ROGERS ET AL.)

EXPOSURE CONDITIONS ppm - 7 Hrs	BLOOD METHANOL (mg/l)	DAILY DOSE (mg/kg)	Ratio of total dose in mice to lethal dose humans (300-1000 mg/kg)
1000	97	819	~1
2000	537	1638	~ 1.6 - 5.5
5000	1650	4095	4 - 14
7500	3178	6142	6 - 20
10000	4204	8190	8 - 27
15000	7330	12285	12 - 41